Objectives: Cilostazol is known to be a selective inhibitor of phosphordiesterase 3 (PDE3) and is generally used as a pharmacological tool in the patients with peripheral arterial disease (PAD). Cilostazol has several beneficial effects, however, little information is available about the angiogenesis. In the present study, we investigated whether cilostazol induces improvement of blood flow in the ischemic hindlimb of mouse, and if so, examined the mechanisms of angiogenesis.

Methods: Wild-type (WT) mice were separated into two groups. One is control group that was treated regular food. The other is cilostazol group that was treated cilostazol as food admixture at a concentration of 0.1%. Ischemic hindlimb model of mice was made by ligation left femoral artery and branched artery. Laser Doppler measurements of blood flow were compared between the control and cilostazol group, on before and postoperative days 0,3,7 and 14. The effect of NOS inhibitor (NG-nitro-L-arginine methyl ester) on cilostazol group was also investigated using Laser Doppler analysis. Capillary density in histological samples of adductor muscle immunostained with an anti-CD31 monoclonal antibody was observed on postoperative day 14. The phosphorylation of endothelial nitric oxide synthase (eNOS) was assessed by Western blot analysis on postoperative day 5. **Results:** In cilostazol group, an angiogenesis by laser Doppler measurements of blood flow ($p<0.05$) and capillary density in adductor muscle ($p<0.05$) in ischemic hindlimb model of mice were significantly enhanced compared to non-treated mice (control), respectively. Cilostazol-induced angiogenesis were significantly inhibited by treatment of NOS inhibitor. Western blot analysis demonstrated that treatment with Cilostazol induced the increasing in eNOS phosphorylation in ischemic tissue.

Conclusions: These results suggest that cilostazol caused angiogenesis in ischemic hindlimb model of mouse. This angiogenesis may be due to the enhancement of nitric oxide production.

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PS230.

A Novel In Vitro Model of Chronic Venous Insufficiency

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Objectives: Chronic venous insufficiency (CVI) is an enormous health burden, affecting 2.5 million patients with advanced disease and leg ulceration per year with costs in the United States alone exceeding $1 billion annually. We developed a novel in-vitro cell culture model to examine alterations in the TGF-β1 pathway, previously shown to be disrupted in human venous ulcers.

Methods: Commercially available healthy, adult dermal fibroblasts (early passage) were used to represent "normal fibroblasts" not yet subjected to the pathologic stretch experienced in CVI. The healthy fibroblasts were incubated on collagen coated plates, and a Flexercell® strain unit set to continuous stretch at 21% above ATM with equibiaxial elongation was used to subject these cells to a pathologic stretch environment for 1, 5 and 7 days. Alterations in TGF-β1 secretion and TGF-β1 Receptor (I and II) expression in response to mechanical stretch were detected by fluorescence activated cell sorting analysis (flow cytometry) and measured by % antibody expression and mean fluorescent intensity. Extracellular (secreted) TGF-β1 was quantified in media using enzyme linked immunosorbent assay and fibroblast migratory capacity was measured using the Boyden chamber transwell migration assay.

Results: TGF-β1 secretion from dermal fibroblasts subjected to 24 hours of stretch was 5-fold less compared to their control (1169 vs 219 pg/mL, $p = 0.05$). Transwell migration was also reduced in stretched cells. A significant reduction in TGF-β1 Receptor I expression (70.4% vs 36.1%, $p = 0.04$) following 5 days of mechanical stretch was also demonstrated.

Conclusions: This model reliably induces reproducible TGF-β1 dysregulation in dermal fibroblasts. Our results support the observation that pathological dermal stretch is associated with and plays an integral role in the disruption of the TGF-β1 pathway present in CVI.

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PS232.

Expression of the Heat Shock Protein HSPB1 Is Associated With Impaired Smooth Muscle Relaxation

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Objectives: The small heat shock protein (HSP27 or HSPB1) has been implicated in regulating vascular smooth muscle tone. HSPB1 is phosphorylated by a stress activated signaling cascade which includes p38 MAP kinase and MAPKAP kinase II (MK2). The purpose of this investigation was to determine if HSPB1 expression correlated with vascular responses in human saphenous vein (HSV).

Methods: Discarded segments of HSV after peripheral/coronary revascularizations were tested in muscle bath. Immunoblotting was performed to determine HSPB1 expression.

Results: Forty percent of veins harvested for aorto-coronary bypass had no functional viability (n = 39). HSPB1 expression and phosphorylation were inversely associated with relaxation in response to sodium nitroprusside (figure 1). Inhibiting HSPB1 phosphorylation by pretreatment with SB203580, a p38 MAPK inhibitor or a cell permeant peptide inhibitor of MK2, MK2i, enhanced relaxation ($p < 0.05$ figure 2). Rat aortic smooth muscle contains low levels of HSPB1; when treated with recombinant HSPB1 with a protein transduction domain allowing cell entry (rPTDHSPB1) led to increased HSPB1 impairing relaxation (figure 3).

Conclusions: Taken together, these studies support a role for HSPB1 in impaired relaxation of smooth muscle.

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PS234.

A Mouse Model of Vascular Grafting

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Objectives: Grafting of surgically transplanted arteries and veins to the arterial circulation is common in cardiovascular practice. Venous grafts degenerate rapidly following implantation with intimal hyperplasia playing an important role. In contrast, arterial grafts have excellent patency rates in the long-term and might be superior to vein grafts. Here we describe arterial and venous grafting to the mouse aorta.

Methods: A model for implantation of vascular grafts was established by transfer of the vena cava from donor animals to the abdominal aorta in recipients. Arterial grafting was performed by transfer of the abdominal aorta from donor mouse to the same site in recipients. Histology, scanning electron microscopy and confocal microscopy were used to study development of intimal hyperplasia and structural changes following transfer. Intravital microscopy was used to study interactions between leukocytes and graft endothelium.

Results: At different time points postop, scanning electron microscopy revealed that venous graft endothelium suffers structural injury whereas arterial grafts show no changes in endothelial integrity. By cross transfer of grafts from Tie2GFP mice that carry fluorescent endothelial cells into WT mice and subsequent confocal microscopy we determined that degenerated endothelium in venous grafts are of a mixed origin with most cells coming from the grafts but with contribution from arterial endothelium and/or circulating endothelial progenitor cells. Intimal hyperplasia was well developed at 28 days. Intravital microscopy showed rolling and recruitment of leukocytes in vein grafts at 28 days and no such interactions were observed in arterial grafts.

Conclusions: We've developed a stable and reproducible mouse model of vascular grafting. We show endothelial injury, intimal hyperplasia and inflammatory recruitment of leukocytes in vein grafts but not in arterial grafts. This animal model will be used in future experiments studying the roles of inflammation in the formation of intimal hyperplasia.

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PS236.

Hydrophilic Surface Treatment of Thin Film Nickel Titanium Reduces Bacterial Biofilm Production Compared to Commercially Available Endograft Materials

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Objectives: Infections of indwelling medical devices remain a source of morbidity and mortality. We have been investigating the use of thin film nickel titanium (TFN) as a novel material to cover stents for a wide array of vascular applications, and have demonstrated that a super hydrophilic surface treatment prevents platelet adhesion and decreases bacterial adhesion. The current study aimed to show that biofilm producing bacteria will be unlikely to form biofilm on super hydrophilic TFN (S-TFN) and that any bacterial adhesion can be more effectively treated with therapeutic doses of antibiotics compared to commercially available endograft materials.

Methods: TFN was created using a sputter deposition technique to a uniform thickness of 5 microns. TFN then underwent surface treatment to create a hydrophilic layer. Bacterial studies were conducted using Staphylococcus Epidermidis strain 35984, a well characterized biofilm producing species. 1cm2 samples of Dacron, ePTFE, Untreated TFN and S-TFN were placed in 10ml tryptic soy broth, inoculated with 107 bacteria, and incubated at 37oC for 24 hours. Samples were then treated with therapeutic doses of rifampin/vancomycin for 24 hours. SEM images were taken both before and after treatment.

Results: SEM imaging studies demonstrated consistently decreased bacterial adhesion on S-TFN compared to all samples with significantly less biofilm deposition. Treatment with antibiotics for 24 hours demonstrated near clearance of bacteria on S-TFN with all other samples having evidence of persistent biofilm.

Conclusions: We demonstrate that the surface properties of S-TFN, namely its negatively charged, super hydrophilic, and ultra smooth surface reduce the likelihood of bacterial adhesion and significantly reduce biofilm deposi-